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(56) Documents cited
US 3806592 A
Martindale, The Extra Pharmacopoeia 29th.Edn. page 940 "Preparations" see "Pancreolauryl Test"

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UK CL (Edition K) A5B BD BLB BNB
INT CL[5] A61K
Online databases: CAS-ONLINE, DIALINDEX (MEDICINE)

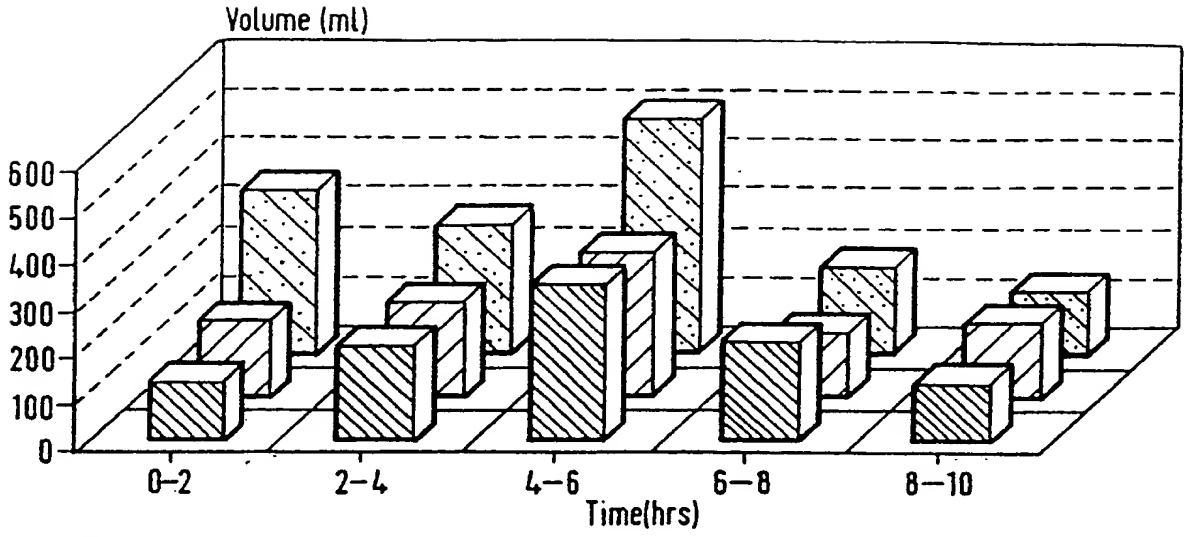
(54) Diagnostic compositions for assessment of pancreatic insufficiency

(57) An emulsified diagnostic composition for detecting pancreatic insufficiency comprises an oil phase dispersed in a continuous aqueous phase, the oil phase having dissolved therein a di-fatty acid ester of fluorescein e.g. fluorescein dilaurate is dissolved in coconut oil and converted into an oil-in-water emulsion in the presence of a mannitol to yield a composition which is administered orally. In the diagnostic test fluorescein and mannitol are excreted in the urine and the ratio of recovered fluorescein and mannitol allows for the detection of pancreatic insufficiency without the need for control experiments.

GB 2 251 381 A

At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

The date of filing shown above is that provisionally accorded to the application in accordance with the provisions of Section 15(4) of the Patents Act 1977 and is subject to ratification or amendment.

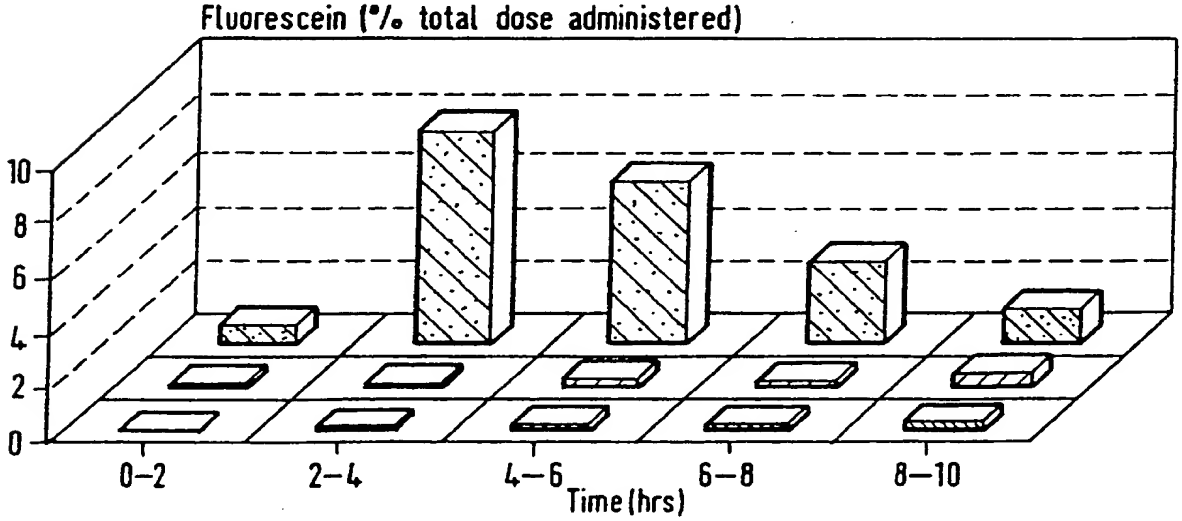


CFW=CF with enzymes

CFWO=CF without enzymes

NORMAL

COMBINED FDL–M TEST:
NORMAL v CF URINARY VOLUMES

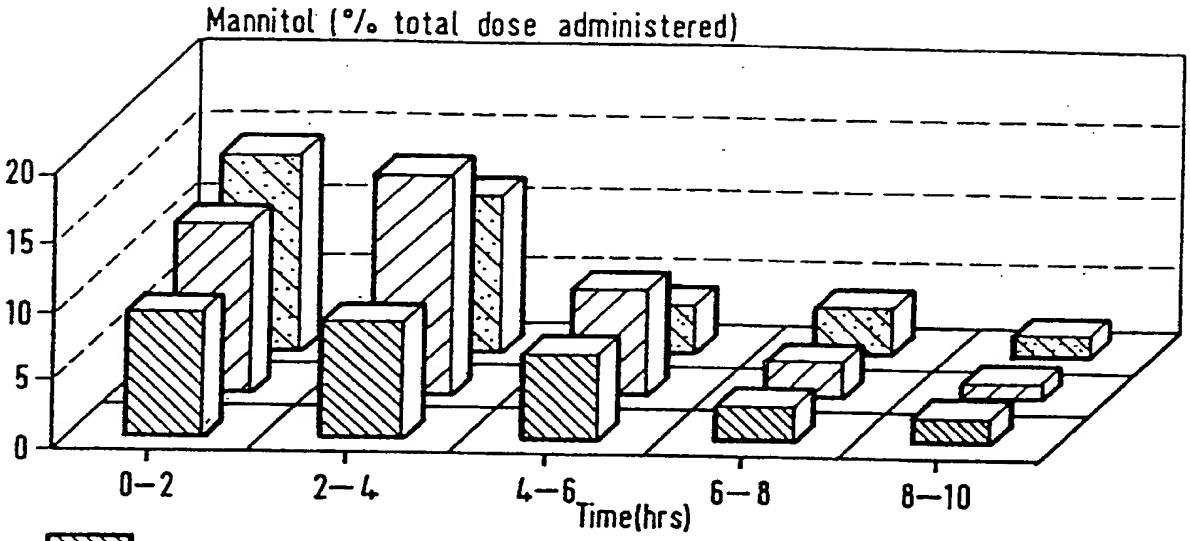


CFW=CF with enzymes

CFWO=CF without enzymes

NORMAL

COMBINED FDL–M TEST:
NORMAL v CF FLUORESCEIN RECOVERY

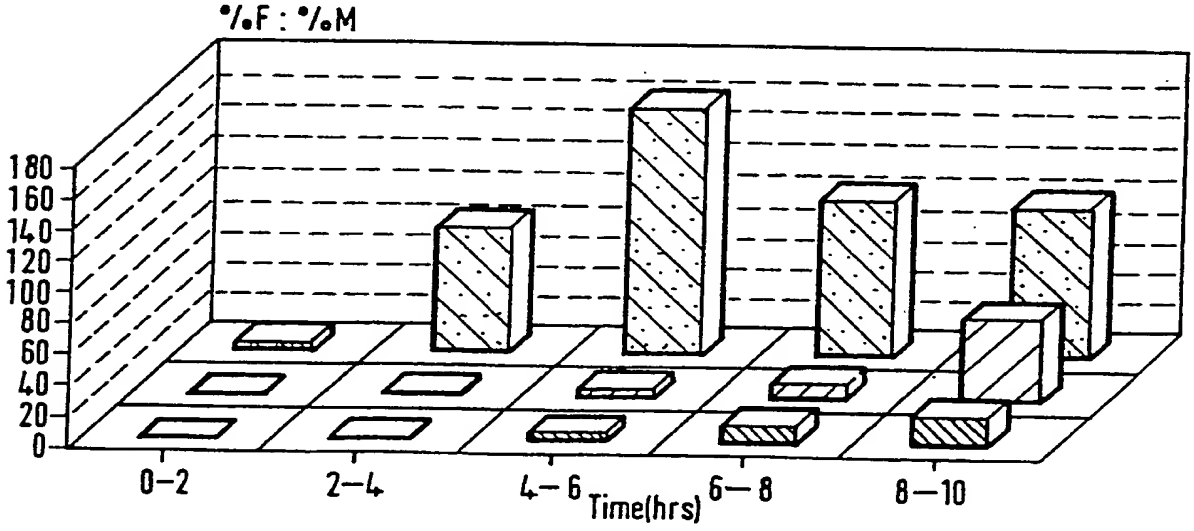


CFW=CF with enzymes

CFWO=CF without enzymes

NORMAL

COMBINED FDL–M TEST:
NORMAL v CF MANNITOL RECOVERY



CFW=CF with enzymes

CFWO=CF without enzymes

NORMAL

COMBINED FDL–M TEST:
NORMAL v CF PERCENTAGE RATIOS

DIAGNOSTIC COMPOSITIONS FOR ASSESSMENT OF PANCREATIC INSUFFICIENCY

This invention relates to diagnostic compositions and method for detecting pancreatic insufficiency.

Identification of pancreatic insufficiency is a useful tool in diagnosis of a variety of diseases, including pancreatitis and cystic fibrosis. One test which has been widely used for routine detection of pancreatic insufficiency involves the administration of fluorescein dilaurate (herein referred to as FDL) When this compound comes into contact with pancreatic juices it is broken down by pancreas-specific cholesterol ester hydrolase into fluorescein and two molecules of lauric acid. The coloured, water-soluble fluorescein is absorbed and excreted in the urine. If the patient's urine is collected over a ten hour period, and the fluorescein content determined, this provides an indirect measure of glandular output of the exocrine pancreas. This procedure is described, for example in Barry et al, The Lancet 1982, Vol. 2, pages 742 to 744.

One disadvantage which is inherent in the above-described pancreolauryl test is that it requires to be carried out over a two day period, since the amount of fluorescein which is excreted must be compared with the amount excreted when an equivalent amount of non-esterified fluorescein is administered to the patient. This control test enables the physician to compensate for

any individual absorption or excretion characteristics which could interfere with the result.

Thus, while effective and suitable for routine diagnostic testing, the pancreolauryl test does have two disadvantages, namely, that it is necessary to carry out a control test and secondly the whole of the urine excreted by the patient after administration of the diagnostic composition must be carefully collected.

One aspect of the present invention is based upon the discovery that by incorporating a particular type of permeability marker in the diagnostic composition, it becomes no longer necessary to carry out a control test, since determination of the marker simultaneously with determination of the fluorescein enables the technician or physician to compensate for differences in absorption of the active materials and excretion of the fluorescein and permeability marker by individual patients.

According to one aspect of the present invention there is provided a diagnostic composition which comprises a di-fatty acid ester of fluorescein and a non-toxic water-soluble substance, such as a saccharide, which is resistant to break-down within the stomach and does not take part in any active transport system across the intestinal mucosa.

Preferably, the di-fatty acid ester is fluorescein dilaurate (hereinafter referred to as FDL), since this is readily broken down by the enzyme present in pancreatic

juices produced by a healthy pancreas. Preferably the saccharide is a saccharide such as mannitol, lactulose, rhamnose, xylose or fructose of which mannitol is most preferred. Mannitol and lactulose have the particular property that they are highly resistant to break down in the stomach and absorption through the intestinal mucosa seems to be substantially passive. In other words, the transport of the saccharide through the intestinal wall does not seem to involve any reaction with any other material in the digestive system.

Preferably, the saccharide, e.g. the mannitol, is present in the diagnostic composition in a molar excess compared with the FDL. Preferably, the molar ratio of FDL to mannitol is from 1:5 to 1:100, more preferably 1:10 to 1:60, e.g. 1:50.

Although various dosage forms of the diagnostic test kits may be used, e.g. encapsulated compositions or tabletted solid compositions, such dosage forms are usually unsuitable for children (including neonates) or indeed for adult patients who have swallowing difficulties. However, because the fluorescein fatty acid ester is water-insoluble, addition of the marker compound to a liquid feed, such as milk, or a watery drink is not possible.

According to a further aspect of the present invention, there is provided an emulsified diagnostic

composition for detecting pancreatic insufficiency which comprises an oil phase dispersed in a continuous aqueous phase, the oil phase having dissolved therein a di-fatty acid ester of fluorescein.

In a preferred form of the invention as applied to liquid formulations, there is provided an emulsified diagnostic composition for detecting pancreatic insufficiency which comprises an oil phase dispersed in an aqueous phase, the oil phase having a di-fatty acid ester of fluorescein dissolved therein and the composition including a saccharide which is resistant to breakdown within the stomach and which does not take part in any active transport system across the intestinal mucosa.

Emulsions prepared in accordance with the invention may be readily added to an infant's drink and an accurate pancreatic insufficiency assessment carried out. In the case of emulsions which additionally contain a saccharide or other metabolic resistant second marker, no control test need, of course, be carried out.

The emulsions are prepared by dissolving the fluorescein di-fatty acid ester (preferably the dilaurate) in a suitable oil phase. Triglycerides have been found suitable, especially those of medium chain length. One specific example is a fractionated coconut oil obtainable under the trade name 'Miglyol 812' from Dynamit Nobel. These dissolve the fluorescein ester without presenting absorption problems to patients suffering from cystic

fibrosis.

Having dissolved the fluorescein di-ester in the triglyceride, an oil-in-water emulsion is formed by dispersing the triglyceride as fine droplets in the aqueous phase. Where a saccharide is present, this may be pre-dissolved in the water. An emulsion is preferably formed by use of ultrasonic energy, although other dispersing methods may be used to obtain emulsion homogenisation. Ultrasonic dispersion has the advantage that an extremely fine dispersion of oil droplets is formed in the aqueous phase. This 'micro-emulsion' system enhances the physical stability of the emulsion, important in paediatric formulations, where the choice of acceptable emulsifiers is limited. Moreover, micro-emulsions require little effort to form an homogenous mixture when added to an infant drink. Suitable stabilising surfactants include lecithin, e.g. soya bean lecithins which are obtainable from Lipoid K.G. and Lucas Meyer under the trade names 'Lipoid 505' and 'Topcithin', respectively.

The emulsions are prepared in dosage unit forms so that a single dosage unit can be added to a standard infant feed, e.g. a 150 ml feed. The fluorescein di-fatty acid ester may be present in an amount of from about 0.1 to 0.5 m.moles, preferably about 0.125 m.moles.

In the case of the emulsions containing a saccharide as the second marker compound, the saccharide is, of course, present to provide a means of measuring gastro-intestinal permeability. The saccharide should be non-metabolisable and the preferred saccharide is mannitol. Alternatives include lactulose, xylose and rhamnose. The saccharide should be present in molar excess compared with the fluorescein ester. Typically, the saccharide is present in the emulsion in concentrations of from 1 to 10 m.moles, e.g. about 6.25 m.moles. The resulting emulsions are filled into boro-silicate vials in the desired unit doses, sealed and autoclaved to sterilise them.

The following specific formulations of emulsion formulations will illustrate the invention. In all cases, the FDL was pre-dissolved in the Miglyol 812, the remaining components were added and emulsified using an ultrasonic disperser.

Example I
Single Marker System

Component	mg/dose	% w/w
Fluorescein Dilaurate	87.00	0.87
Miglyol 812 (coconut oil triglyceride)	1250.00	12.50
Lipoid S05 (soya bean lecithin)	250.00	2.50
Water (mains, boiled)	8413.00	84.13
	10000.00	100.00

Example 2
Dual Marker System

Component	mg/dose	% w/w
Fluorescein Dilaurate	87.00	0.87
Mannitol B.P.	1138.00	11.38
Miglyol 812	1250.00	12.50
Lipoid S05	250.00	2.50
Water (mains, boiled)	7275.00	72.65
	10000.00	100.00

As stated above, the diagnostic composition may be administered as an encapsulated solid composition or a tabletted composition which may be administered in one or more tablets or capsules. Typically, a dosage unit of the FDL is from 0.2 to 0.8 mmols and the mannitol from about 20 to 30 mmols with FDL and mannitol being typically in the moler ratio of 1:50. The FDL and Mannitol may be intimately mixed to form a homogeneous powder and filled into hard gelatin capsules. Suitable flow and compaction aids, commonly known to those familiar to capsule technology, may be added to assist production.

An example of an encapsulated formulation is as follows:

Component	mg/dose	% w/w
Fluorescein Dilaurate	87.00	7.03
Mannitol	1138.00	91.97
Aerosil 200 (colloidal silicon dioxide)	12.25	1.00
	1237.25	100.00

The powder mix may be filled into two/three size 00 hard gelatin capsules.

The formulation illustrated above is intended for infants and is one-quarter of the standard adult does. A standard adult dose would therefore be 0.5m Moles (348.5 mg) of FDL and 25m Moles (4552.0 mg) of Mannitol contained in the appropriate number of capsules.

It has been found that after administration of the diagnostic composition a steady state is reached after about 4 to 6 hours from administration, at which time a sample may be taken and analysed for fluorescein and mannitol contents. The ratio of fluorescein to mannitol in the sample gives a clear discrimination between normal pancreatic activity and pancreatic insufficiency.

Fluorescein may be conveniently analysed quantitatively in a sample using a spectrophotometer. The evaluation can be carried out directly by measuring the absorption of a sample of urine containing the excreted fluorescein and mannitol. In the case of mannitol determination, this is conveniently carried out by the method described in the paper by Lunn et al, published in Clinica Chimica Acta, 1989, CCA04504. In this method, mannitol is treated with mannitol dehydrogenase which is extracted from a culture of a bacterium NCIB NCTC 6992, obtainable from NCIB Torry Research Station, Aberdeen Scotland. This particular enzyme converts mannitol into

fructose and NADH which can be determined by optical density measurements at 340 nm. The relationship between the original mannitol concentration and the optical density of a sample treated with the bacterium is almost exactly linear, so that the optical density can be calibrated in mmols of mannitol. Thus, samples of the excreted urine after administration of the diagnostic composition can be determined for fluorescein and mannitol concentrations by a simple spectrophotometric analysis before and after addition of the enzyme which converts the mannitol into fructose.

In order to demonstrate the operation of the diagnostic method, the following study was carried out of 6 healthy adults (four male and two female) aged 29 to 40 years which constituted group 1. Six patients diagnosed as having cystic fibrosis (five male and one female aged 7 to 21 years) were tested both before having been supplied with pancreatic enzyme supplement (group 2) and after having been supplied with such a supplement (group 3).

After an overnight fast, each of the patients ingested an encapsulated composition comprising 0.5 mmols of FDL and 25 mmols of mannitol at time zero followed by a standardised breakfast consisting of 50 gms of bread spread with 20 gms of butter and 1 cup of liquid.

Urine was collected for ten hours in 2 hourly aliquots and urinary fluorescein was measured photometrically and mannitol was measured enzymatically

and photometrically as described above. There was no significant difference in mean (standard deviation) ten hour urine volume which were 1475 (278 mmls) group 1, 1008 (398 mmls) group 2 and 849 (536 mmls) for group 3. There was also no significant difference in percentage mannitol recovery 35.0 (11.1%) group 1, 40.1 (18.1%) group and 28.6 (7.0%) group 3. However, there was a pronounced difference in percent fluorescein recoveries between the three groups, i.e. 18.7 (3.8%) group 1, 1.2 (0.5 %) group 2 and 0.8 (0.3%) group 3, resulting in a significant difference in ten hourly fluorescein to mannitol ratios 57.3 (18.2). 3.4 (1.4), 3.1 (1.9) respectively.

These results are shown schematically in the attached bar charts. When the results were analysed in two hour urine aliquots it was seen that there was a delay in urinary recovery of fluorescein versus mannitol which is due to the pancreatic digestive phase in both controls and cystic fibrosis groups. Differences in urinary fluorescein to mannitol ratios between controls and cystic fibrosis patients (both those with and without pancreatic supplements) achieved biological and statistical significance from 2 to 8 hours post ingestion of the diagnostic composition. These results show that the addition of mannitol to FDL permits clear distinction between subjects with normal pancreatic function and those with cystic fibrosis whether receiving pancreatic enzyme

supplements or not. Thus, by using the new diagnostic test it is possible to detect pancreatic insufficiency by taking samples between 2 and 8 hours after ingestion of the composition without the need to run a further control.

CLAIMS:-

1. An emulsified diagnostic composition for detecting pancreatic insufficiency which comprises an oil phase dispersed in a continuous aqueous phase, the oil phase having dissolved therein a di-fatty acid ester of fluorescein.

2. A composition according to claim 1 which also contains a saccharide which is resistant to breakdown in the stomach and does not take part in any active transport system across the intestinal mucosa.

3. A composition according to claim 2 in which the fluorescein ester and the saccharide are present in a molar proportion of about 1:5 to about 1:100, preferably from about 1:10 to about 1:60.

4. A composition according to any one of the preceding claims which is in the form of a concentrate intended for addition to a liquid feed and wherein the fluorescein ester in present in the emulsion in a concentration of from about 0:1 to 0.5 m.moles.

5. A composition according to any one of the preceding claims in which the saccharide is present in a concentration of from about 1 to 10 m.moles.

6. A composition according to any one of the preceding claims in which the fluorescein ester is fluorescein dilaurate.

7. A composition according to any one of claims 2 to 6 in which the saccharide is mannitol, lactulose, xylose or rhamnose .

8. A composition according to any one of the preceding claims in which the fluorescein is dissolved in a triglyceride.

9. A composition according to claim 8 in which the triglyceride is a medium chain length triglyceride.

10. Use of a composition comprising an emulsion, containing a di-fatty acid ester of fluorescein and a saccharide which is resistant to breakdown within the stomach and does not take part in any active transport system across the intestinal mucosa, in the assessment of exocrine pancreatic function.

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Patents Act 1977
Examiner's report to the Comptroller under Section 17 (The Search Report)

Application number

9126224.6

Relevant Technical fields

(i) UK Cl (Edition K) A5B (BD,BLB,BNB)

(ii) Int Cl (Edition 5) A61K

Search Examiner

J F JENKINS

Databases (see over)

(i) UK Patent Office

(ii) ONLINE DATABASE: CAS-ONLINE, DIALINDEX (MEDICINE)

Date of Search

26 FEBRUARY 1992

Documents considered relevant following a search in respect of claims 1 TO 10

Category (see over)	Identity of document and relevant passages	Relevant to claim(s)
A	Martindale, The Extra Pharmacopocia 29th Edn. page 940 "Preparations" see "Pancreolauryl Test"	1

SF2(p)

sf - c:\wp51\doc99\fil001979

Category	Identity of document and relevant passages	Relevant to claim(s)

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